Journal of Ornamental Plants
Available online on: www.jornamental.iaurasht.ac.ir
ISSN (Print): 2251-6433 ISSN (Online): 2251-6441

The Effects of Nitrogen Fertilizer Sources on Yield and some Quality Attributes of *Chrysanthemum morifolium* L.

Lamia Vojodi Mehrabani
Department of Agronomy and Plant Breading, Faculty of Agriculture, Azarbaijan Shahid Madani University, Tabriz, Iran

Received: 16 February 2017 Accepted: 16 September 2017
*Corresponding author's email: vojodilamia@gmail.com

Abstract

Nitrogen is a crucial nutrient affecting plant growth and development and its addition to the soil (as chemical or organic form) influences plant growth characteristics. One experiment was conducted as a factorial based on RCBD with two *Chrysanthemum* cultivars ('Fred' and 'Angel'(and two N-fertilizer sources [Urea (75 and 150 kg /h and Nitroxin 7.5 and 15 L /ha] used as fertigation method beside control treatment with three replications. The results revealed that the greatest petals carotenoids content was belonged to 15 liter/ha Nitroxin and the least data for total phenolics was recorded in the control treatment with 'Angel'. Urea at 150 kg / ha increased the total phenolics content. The high chlorophyll a content was obtained with Nitroxin (15 L / ha) with 'Fred' and Urea (150 kg /h) with 'Angel'. The least chlorophyll b content was recorded with 150 kg / ha Urea at 'Angel'. Roots dry weight (15 L /ha Nitroxn), stem dry weight (15 L Nitroxin and 150 kg /ha Urea) were positively affected in 'Fred'. Fertilizers application positively affected root fresh weight in both cultivars. The highest content of anthocyanin (19.3 µmol g^{-1} FWt) was recorded in 'Angel' cultivar. Anthocyanin content was also influenced by 150 kg / ha Urea (19.2 µmol g^{-1} FWt) and 15 L/ha Nitroxin (18.4 µmol g^{-1} FW). The highest amount of shoot number (12.6) was recorded in 'Fred' cultivar. With any increase in the fertilizer levels, flowers fresh weight and shoot numbers were significantly increased.

Keywords: Anthocyanin, *Chrysanthemum morifolium*, Nitroxin, Phenolic, Urea.

INTRODUCTION

Chrysanthemum morifolium is a perennial of the Asteraceae family (Ze-lin and Harnly, 2010). The flowers have widespread applications in traditional medicine as drinks as well as they have been consumed as vegetables in some countries like China, Japan and Thailand. Pharmacological studies have demonstrated that these plants are containing secondary metabolites such as flavonoids, alkaloids, phenolics, and terpenoids with a wide range of biological activites. These components have anti-bacterial, anti-fungal, anti-inflammatory, anti-pain and antioxidant properties (Park *et al.*, 2015). *Chrysanthemum morifolium* is an important cut ornamental species worldwide as well. Besides above, the plant attracts more attention of landscape designers due to the great diversity in flower shape, color and scent. Flower color in the plant is dependent upon the agglomeration of secondary metabolites such as flavonoids, carotenoids and β-alanine (Zeng *et al.,* 2014). Anthocyanins are the reason for the red, pink and blue colors of the petals, the dominant insect pollinators and also protect the plants from UV radiation and diseases (Bakhshi and Arakawa, 2007). Owing to the long-lasting flower formation, *Chrysanthemum* cultivars are a nice source for the extraction of phenolics, carotenoids and anthocyanin compounds (Ze-lin and Harnly 2010; Park *et al.*, 2015). Flavonoids profile in the diverse tissues of the plant is quite different and varies significantly between cultivars (Mejla *et al.*, 1981). Due to the ornamental and medicinal properties of this plants, the interest in cultivation and utilization of the plants and its preparation has been increased dramatically. However, over-using of chemical fertilizers in the production of this plant has imposed several environmental restraints and leads to increased input charges, soil deterioration and organic matter reduction, the scarcity of minerals as well as the susceptibility of the plants to pest and diseases and also greatly influences a soil fauna (Bashyal *et al.*, 2011). Using bio-fertilizers as environment friend compounds reduces the majority of the above mentioned problems and greatly reduces input and production charges. Bio-fertilizers are organic amendments containing one or several organisms or their metabolites which provide some major essential minerals and organics needed for plants normal growth and development under a sustainable agronomic production system.

Nitrogen is the most important mineral commonly absorbed as NO_3^- or NH_4^+ by the plants (Selvakumar *et al.,* 2009). Nitrogen effects on plant growth and development is in main part due to its dominant role in protein biosynthesis and photosynthesis rate. Nitrogen bio-fixation is an alternative way hugely reduces chemical N fertilizer input and at the same time prevents soils organic matter loss and also greatly reduces environmental pollution (Selvakumar *et al*., 2009). Heidari *et al.,* (2014) noted that bio-fertilizers application significantly increased the chlorophylls, protein and total carotenoids content in saffron. *Chrysanthemum morifolium* is a suitable candidate for mass production under Northwest Iranian climatic condition mainly due to its high adaptability and low input needs. This experiment was conducted to study the effects of bio-fertilzers Nitroxin compared to urea on some morph-physiological traits of two *Chrysanthemum morifolium* cultivars under the semi-dry climatic Northwest Iranian climactic conditions.

MATERIALS AND METHODS

This experiment was conducted as factorial based on RCBD with two *Chrysanthemum morifolium* cultivars, 'Fred' (white petals) and 'Angel' (purple petals). Two nitrogen sources Urea (75, 150 kg/h) and Nitroxin (7.5 and 15 L/ha) as fertigation plus control plants were employed. Two weeks after planting, the cuttings (having 3-4 leaves) were transferred to 5 liter pots containing common soil. (Loam soil with pH: 7.5, EC: 1.72 dS/m and N: 0.16 %). The fertilizer treatments were divided to apply for three times with one month intervals. The leaves (shade dried for one week), flowers and root samples were dried at the ambient laboratory conditions (shade dried at 20-27 °C for two weeks) (Hassanpouraghdam *et al.*, 2010). Later, morpho-physiological traits were assayed.

Measurements

Growth parameters

Plant dry weight, shoot number, plant length, flower dry weight, flower number and diameter were recorded.

Chlorophyll content

Chlorophyll content was measured by the method described by Prochazkova *et al*., (2001) spectrophotometrically.

Total phenolics, flavonoids and anthocyanins content

Total phenolic content was assayed by Folin-ciocalteu by a methods described by Ghorbanli *et al.* (2011) as mg gallic acid per g of plant dried weight. Total flavonoids were quantified by $AlCl_3$ as the methods of Ghorbanli *et al.* (2011) based on mg Rutin per g dry weight of plant material. Total anthocyanin were quantified by Wanger (1979).

Petal carotenoids content

100 ml acetone was added to 30 grams of dried petals. The flask was shaken for two minutes. The extracts were diluted as 1:100 with acetone again. After shaking, the absorbance was read at 462 nm by spectrophotometer (Gomez-Garcia Mdel and Ochoa-Alejo, 2013).

Essential oil content

The essential oil extraction was done from 30 g dry plant material by water distillation during 3 hrs using a Clevenger-type apparatus and the oils were dried over anhydrous sodium sulphate (Zeng *et al.*, 2014; Ghorbanli *et al.,* 2011).

Data analysis

The data were analysed by MSTATC and SPSS. Mean comparisons were done by LSD.

RESULTS AND DISCUSSION

ANOVA showed the significant interaction effects of cultivars and the fertilizer treatments on total phenolics, carotenoids, chlorophyll a and b, root and stem fresh and dry weight, flower number and plant height (Table 1 and 2).

The effects of cultivars and N source on leaves, stems and roots fresh and dry weight

The greatest root and stem dry weight was belonged to 15 liter/ ha nitroxin at ‘Fred’. The highest data for root fresh weight was recorded at 150 kg/h urea and 15 liter/ ha nitroxin with ‘Angel’ and 15 liter nitroxin with ‘Fred’ (Table 3). The least root fresh weight was attained by control ‘Angel’ plants (Table 3). It seems that root dry weight reduction in control plants is due to low nitrogen availability. Biyari *et al.*, (2007) reported the same reason i.e. N leaching and low availability of N was the main factor for reduced maize plant growth. N is the major mineral and nutrition element affects vegetative growth and leaf area expansion in plants. Under N deficiency, leaf growth and leaf area index declines drastically. This in main part is due to reduced net photosynthesis rate and or restricted cell expansion (Heidari *et al.*, 2015). The positive effects of bio-fertilizers containing *Azotobacter* and *Azospirillum* on root fresh and dry weight, root length and flower number have been reported in different plants (Bashyal, 2011). Their positive growth promoting effects is due to the macro and micro nutrients availability, N_2 fixation, the production of growth promoting substance like auxins, cytokinins and gibberellns, as well as colonization with micoririza fungi and the low incidence of diseases (Lugtenberg and Kamilova, 2009). Heidari *et al.*, (2015) in saffron reported that the combined use of 50 Kg of N+ bio-fertilizers increased picrocrocin content in stigmas and also raised yield and other quality attributes. Prabhatkumar and Mishra (2003)

Table 1. ANOVA for the effects of Urea and Nitroxin on some morpho-physiological traits of Chrysanthemum morifolium.

S.o.V	df	Shoot number	Plant length	Flower number	Flower dry weight	Chl. a	Chl. b	Total phenolic content	Flavonoid content	Anthocyanin content	petals carotenoids content	Essential oil content
Replication	2	3.94^{ns}	13.77^{ns}	194.54*	23.42^{ns}	0.008^{ns}	0.34*	21.3^{ns}	25.26^{ns}	151.40**	0.02^{ns}	2.65^{ns}
Cultivar (C)	1	23.84**	14.2^{ns}	44.89^{ns}	25.79^{ns}	0.148^{ns}	0.006^{ns}	1649**	61.40^{ns}	1040.4**	0.44**	2.60^{ns}
Treatment (T)	5	89.57**	187**	292.62**	26.39^{ns}	0.605**	0.39*	5692**	163.9**	125.75**	0.12**	4.65^{ns}
T× C	5	2.01^{ns}	32.5**	321.77**	24.61^{ns}	0.139**	0.36*	85.48**	75.3^{ns}	18.60^{ns}	0.052*	2.78^{ns}
Error	22	1.13	5.76	53.83	24.15	0.014	0.107	26.71	31.70	24.52	0.019	2.79
C.V. (%)		8.99	8.24	26.87	44.46	4.71	24.92	6.96	27.65	35.43	39.1	17.94

ns,* and ** show non-significant and significant at P<0.05 and P<0.01, respectively.

Table 2. ANOVA for the effects of Urea and Nitroxin on some morpho- physioloigical traits of Chrysanthemum morifolium.

S.o.V	df	Flower diameter	Flower fresh weight	Stem fresh weight	Root fresh weight	Flower dry weight	Stem dry weight	Root dry weight
Replication	2	0.94**	0.034^{ns}	56.13*	1.77^{ns}	28.02^{ns}	2.88^{ns}	0.28^{ns}
Cultivar (C)	1	0.80^{ns}	0.003^{ns}	751.0**	7.54^{ns}	30.73^{ns}	638.4**	12.32**
Treatment (T)	5	0.83*	0.66**	915.13**	472.83**	31.39^{ns}	148.0**	49.76**
T× C	5	0.21^{ns}	0.008^{ns}	141.25**	60.42**	29.63^{ns}	23.39**	6.66**
Error	22	0.19	0.068	13.84	4.89	29.02	4.15	0.98
CV (%)		11.21	10.92	7.89	8.57	41.26	0.18	12.92

ns,* and ** show non significant and significant at P<0.05 and P<0.01, respectively.

reported that bio-fertilizers application in China aster influenced the plants productivity by increased vegetative growth, accelerated hairy root formation, elevated minerals acquisition, chlorophylls biosynthesis, carbohydrates accumulation and improved production of psedo-hormones. In total, *Azotobacter* included in bio-fertilizers, improves yield by the availability of molecular nitrogen and also by the impact they have on the absorption of N, P, K, Fe and Zn as well as by the improved water uptake. Moreover, bio-fertilizers accelerate nitrate reductase activity and stimulate the biosynthesis of growth promoting substances.

Effects of cultivar and N fertilizer on shoot number

Table 4 showes that with 15 liter/ha nitroxin and 150 kg /ha urea, the shoots number increased. Zahir *et al*. (2004) in their studies demonstrated that indole-3- acetic acid production by *Azotobacter* strains was the predominant reason for promoting growth characteristics. Vessey (2003) reported that the production of auxin and GA_3 by *Azospirillum* was the main reason for improved growth and development of plants. These plant growth regulators impact cell division and enlargement and hence organ development (Vassey, 2003). By this way, these bacteria will improve the plant height, stem diameter, leaf number, and finally plants yield and productivity.

The effects of N source on the flower number, weight and diameter

The least flower diameter was belonged to control and 75 kg / ha of urea. Table 4 shows that with 1.5 liter nitroxin and 150 kg / ha urea, the flowers fresh weight was increased. These are in line with the findings of Agha-Alikhani *et al.* (2014) in Echinacea purpurea; where, they reported the increased soil microbial biomass, and the production of growth regulators and the high availability of nutrients such as N and P in the plants treated with bio-fertilizers like nitroxin and biophosphorous.

Chlorophyll, carotenoid and essential oil content

The highest petals carotenoids content was belonging to 'Angel' at 15 liter nitroxin (Table 3). For chlorophyll b, the lowest data was attained by 150 kg / ha of urea in 'Angel'. The highest chlorophyll a was measured by 1.5 liter nitroxin in 'Fred' and 150 g L^{-1}/ ha urea in 'Angel' (Table 3). Jafarzadeh *et al.* (2014) reported that the optimized flower yield and photosynthetic pigments content in *Calendula officinalis* was obtained by nitroxin applying as seed coat. The essential oil content of the flowers was not affected by the treatments (Table 1).

Phenolics, flavonoids and anthocyanin content

The highest total phenolics for both cultivars were belonged to 150 kg / ha of urea (Table 3). For flavonoids, the same treatment was the best as well. Mean comparison also revealed that, with increasing bio-fertilizer levels, i.e. Nitroxin (15 L^{-1} /ha) and urea (150 kg / ha) anthocyanin content was increased and 'Angel' had more anthocyanin content than 'Fred' (Table 5). Ibrahim *et al*. (2011) reported that in *Labisia pumla* Blume, phenolics and flavonoids content was the greatest with 90 kg/ha of nitrogen fertilizers application. The main reason for was related to PAL activity. AghaAlikhani *et al.* (2014) noted that in *Echinacea purpurea*, bio-fertilizers improved the yield attributes. In another study, Park *et al.* (2015) demonstrated that there were significant differences between cultivars considering pigments content. 'Angel' was containing the highest anthocyanin and carotenoids content (Table 3). Our findings are in line with the finding of Nader-Nejhad *et al.* (2014) whom reported that cultivar type had prominent effects on the phenolic content of pistachio fruits. Phenolics are strong antioxidants capable of scavenging ROS molecules and so prevent the oxidation of vital metabolites and could be used as antioxidant reagents in food and pharmaceutical industries (Park *et al.,* 2015).

Table 3. Mean comparison for the interaction effects of cultivar and fertilizer treatment on some morpho- physioligical traits of *Chrysanthemum morifolium*.

Cultivar	Fertilizer	Root fresh weight (g)	Stem dry weight (g)	Root dry weight (g)	Chl. a (mg g^{-1} FWt)	Chl. b (mg g^{-1} FWt)	Phenolic content (mg GA g^{-1} DWt)	Petal carotenoids contents (%)
'Fred'	Control	13.43 e	10.83 de	3.36 d	1.52 f	0.81 b	15.59 f	0.011 i
'Fred'	7.5 Nitroxin	29.48 b	20.7 b	10.10 b	2.31 cd	1.59 a	13.13 e	0.036 bcd
'Fred'	15 Nitroxin	37.0 a	25.17 a	12.53 a	2.74 a	0.82 b	22.81 cd	0.026 bcd
'Fred'	75 Urea	17.37 d	15.3 c	6.23 c	2.30 cd	1.60 a	15.90 e	0.030 bcd
'Fred'	150 Urea	29.40 b	24.53 a	9.30 b	2.40 cd	1.19 ab	27.83 ab	0.011 d
'Angel'	Control	12.47 e	6.13 f	3.56 d	2.10 e	0.92 b	14.57 f	0.02 cd
'Angel'	7.5 Nitroxin	23.37 c	5.86 f	6.36 c	2.38 cd	1.2 ab	28.27 bc	0.05 b
'Angel'	15 Nitroxin	34.07 a	15.10 c	9.10 b	2.51 bc	1.59 a	19.80 d	0.08 a
'Angel'	75 Urea	26.70 bc	9.03 ef	6.36 c	2.30 cd	1.35 ab	17.53 d	0.04 bc
'Angel'	150 Urea	35.03 a	14.27 cd	9.73 b	2.68 ab	0.79 c	28.90 a	0.04 b
LSD 5%		3.79	3.51	1.70	0.18	0.56	8.82	2.36

Similar letters in the columns are non-significant based on LSD test (P<0.05).

Table 4. Mean comparison for the effects of Urea and Nitroxin on some morpho- physiological traits of *Chrysanthemum morifolium.*

Treatment	Shoot number	Flower diameter (cm)	Flower fresh weight (g)	Anthocynin (μmol g^{-1} FWt)	Flavonoid (mg Rutin g^{-1} DWt)
Control	5.25c	3.46b	1.83c	9.05c	14.82b
0.75 Nitroxin	12.43b	3.98ab	2.29b	12.56bc	20.70b
1.5 Nitroxin	14.20a	4.48a	2.73a	18.42ab	21.31b
75 Urea	12.25b	3.75b	2.39b	11.00c	16.72bc
150 Urea	15.05a	3.82ab	2.58ab	19.22a	28.37a
LSD 5%	1.25	0.53	0.31	5.68	6.83

Similar letters in the columns are non-significant based on LSD test (P<0.05).

Table 5. Mean comparison for the effects of cultivar on some traits of *Chrysanthemum morifolium.*

Cultivar	Anthocyanin (µmol g^{-1} FWt)	Shoot number
'Fred'	8.19b	12.68a
'Angel'	19.37a	10.44b
LSD%	3.28	0.73

Similar letters in the columns are non-significant based on LSD test (P<0.01).

CONCLUSIONS

The great interest in ornamental and medicinal plants usage makes reasonable their worldwide production mainly for their applications in food, hygienic and therapeutic industries. The biosynthesis of secondary metabolites in plants is dependent on the type and amounts of fertilizers applied. In our experiment, N source and amount had significant effects on morphological traits, yield and some metabolites of *Chrysanthemum*. Cultivars type and fertilizer treatments had meaningful effects on total phenolics, carotenoids and chlorophyll a and b contents. Stem and root fresh and dry weight, flower diameter and plant height were influenced by the treatments as well. *Chrysanthemums* are highly adaptive to Northwest Iranian climatic conditions and hence, the mass production of their phenolics is of great interest of the related industries. Considering, management of the nitrogen fertilizer inputs is a major trend to increase the yield and productivity of *Chrysanthemum* cultivars. Doing this, we would be able to meet the increasing demands for these high valued metabolites, to reduce the high production costs and mainly to add up the farmers and producers income.

Literature Cited

Agha Alikhani, M., Iranpor, A. and NaghdiBadi, H. 2013. Change in agronomical and phytochemical yield of purple coneflower (*Echinaceae purpurea* L. Moench under urea and three bio-fertilizer application. Journal of Medicinal Plants, 46 (2): 121-136. (In Persian).

Bakhshi, D. and Arakawa, O. 2007. Induction of phenolic compounds biosynthesis with light irradiation in the flesh of red and yellow apples. Journal of Applied Horticulture, 8(2): 101-104.

Bashyal, L.N. 2011. Response of cauliflower to nitrogen fixing biofertilizer and graded levels of nitrogen. Journal of Agricultur and Environment, 12: 41-50.

Biyari, A., Gholami, A. and Asadi Rahmani, H. 2007. Sustainable production and improvement of nutrient absorption by maize in response to seed inoculation by PGPR. Proceedings of the 2nd National Conference on Agroecology in Iran, Gorgan, Iran. p: 8.

Ghorbanli, M., Saadatmand, L. and Niakan, M. 2011. Study the effects of natural habitats on flavonoids polyphenols, anthocyanin and their related antioxidant activity in *Elaeagnus agustifolia*. The First Congress on Advanced Agricultural Finding, Islamic Azad University Saveh, Iran. (In Persian).

Gomez-Garcia Mdel, R. and Ochoa-Alejo, N. 2013. Biochemistry and molecular biology of carotenoid biosynthesis in chili peppers (*Capsicum* spp.). International Journal of Molecular Sciences, 16:14 (9): 19025-53.

Hassanpouraghdam, M.B., Hassani, A., Vojodi Mehrabani, L. and Farsad, N. 2010. Drying methods affects essential oil content and composition of basil (*Ocimum basilicum* L.). Journal of Esserntial Oil Bearing Plants, 13 (6): 759-766.

Heidari, Z., Besharati, H. and Maleki Farahani, S. 2014. Effect of some chemical fertilizer and biofertilizer on quantitative and qualitative characteristics of saffron. Saffron Agronomy and Technology, 2 (3): 177-189.

Ibrahim, M.H., Jaafar, H.Z.E., Rahmat, A. and Abdul Rahman, Z. 2011. Effects of nitrogen fertilization on synthesis of primary and secondary metabolites in three varieties of kacip fatimah (*Labisia*

pumila Blume). International Journal of Molecular Sciences, 12 (8): 5238–5254.
Jafarzadeh, L., Heshmat, O. and Bostan, A.A. 2014. The study of drought stress and bio fertilizer of nitrogen on some biochemical traits of marigold medicinal plant (*Calendula officinalis* L.). Journal of Plant Research, 27 (2): 180 – 193. (In Persian).
Lugtenberg, B. and Kamilova, F. 2009. Plant-growth promoting rhizobacteria. Annual Review of Microbiology, 63: 541-556.
Mejla, A., Hudson, E., Gonzalez de Mejla, E. and Vasquez, F. 1981. Carotenoid content and vitamin A activity of some common cultivars of Mexican peppers (*Capsicum annuum*) as determined by HPLC. Journal of Food Science, 53: 1448-1451.
Nader-Nejad, N., Ahmadi Moghadam, A., Hosseinifar, J. and Pourseiedi, Sh. 2014. The effects of rootstock and cultivar on PAL, phenolics and flavonoids content in flower, leaves and fruits of pistachio. MSc Thesis, Shahid Bahonar University of Kerman, Iran.
Park, C.H., Chae, S.C., Park, S.Y., Kim, J.K., Al-Dhabi, N.A. and Park, S.U. 2015. Anthocyanin and carotenoid content in different cultivars of chrysanthemum flower. Molecules, 11090-11102.
Prabhatkumar, R.S. and Mishra, R.L. 2003. Effect of bio-fertilizers on growth and yield of China aster. Journal of Ornamental Horticulture, 6 (2): 85-88.
Prochazkova, D., Sairam, R.K., Srivastava, G.C. and Singh, D.V. 2001. Oxidative stress and antioxidant activity as the basis of senescence in maize leaves. Plant Science, 161: 765-771.
Selvakumar, G.M., Lenin, P. and Ravimycin, T. 2009. Response of biofertilizers on the growth and yield of blackgram (*Vigna mungo* L.). Recent Research in Science and Technology, 1 (4): 169-175.
Vessey, J.K. 2003. Plant growth promoting rhizobacteria as biofertilizer. Plant and Soil, 255: 271 – 286.
Wanger, G.J. 1979. Contact and vacuole extra vacuole distribution of neutral sugar free amino acids and anthocyanin in protoplast. Plant Physiology, 64: 88-93.
Zahir, Z.A., Arshad, M. and Frankenberger, W.F. 2004. Plant growth promoting rhizobacteria: Applications and perspectives in agriculture. Advances in Agronomy, 81: 97 - 168.
Ze-lin, L. and Harnly, J.M. 2010. Identification of the phenolic components of chrysanthemum flower (*Chrysanthemum morifolium* Ramat). Food Chemistry. 120 (1): 319-326.
Zeng, Y., Deng, M., Lv, Z. and Peng, Y. 2014. Evaluation of antioxidant activities of extracts from 19 Chinese edible flowers. Published online 2014 Jun. 25. doi: 10.1186/2193-1801-3-315.

How to cite this article:
Vojodi Mehrabani, L. 2017. The effects of Nitrogen fertilizer sources on yield and some quality attributes of *Chrysanthemum morifolium* L. *Journal of Ornamental Plants, 7(4), 229-236.*
URL: http://jornamental.iaurasht.ac.ir/article_537040_169d4e7cba171b1c38c7061a1076f967.pdf